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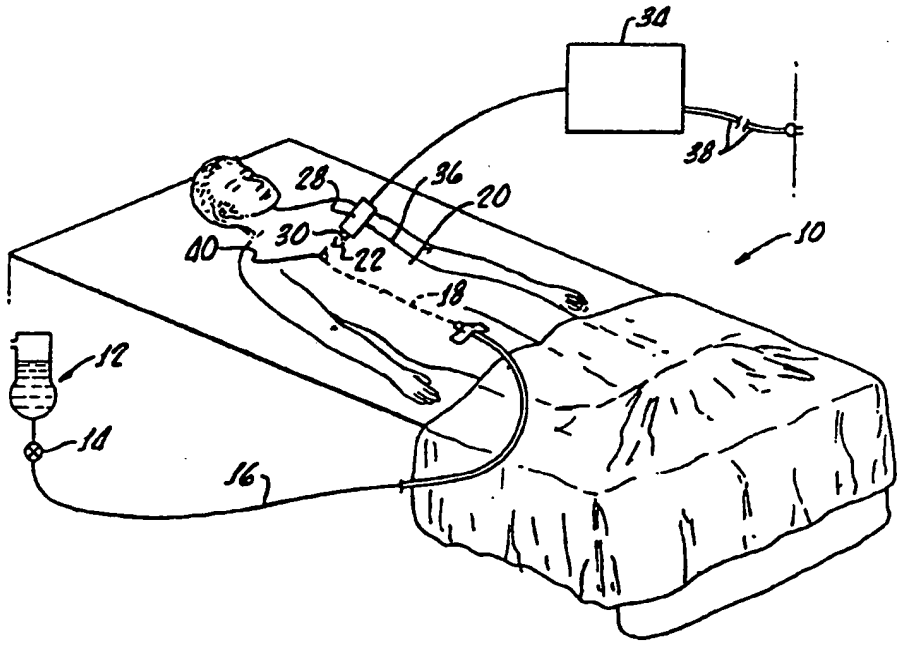

(57) Abstract

Apparatus and methods are provided for utilizing a combination of ultrasonic energy and a microbubble medium for substantially dissolving blood clots or other vascular obstructions.

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ENHANCEMENT OF ULTRASOUND THROMBOLYSIS

The present invention is generally related to the use of ultrasonic energy and is more particularly directed to the use of ultrasound with ultrasound imaging agents to dissolve arterial thrombi.

It is known that ultrasound imaging can be used to locate intravascular thrombi and it has been demonstrated that the utilization of ultrasonic waves can improve the diffusion and penetration of medicinal fluids or the like into the vascular system (see U.S. Patent No. 5,197,946 to Tachibana). Tachibana teaches that in order to effectively enhance or improve the diffusion and penetration of a medicinal fluid, the oscillating element must be disposed at the point of injection of the medicinal fluid.

This is to be contrasted, according to Tachibana, with prior art techniques which utilize ultrasonic waves and a catheter wire for diffusion and penetration of medicinal fluids. In this arrangement, the ultrasonic oscillating element is located outside the body and far from a radiating end of the catheter wire. This results in a significant reduction in efficient coupling of the ultrasound due to the damping of ultrasonic energy in the course of transmission down the catheter wire.

Other disadvantages in the use of a transmission wire to deliver ultrasonic energy to a thrombosis is transmission wire stiffness. Further, as the transmission wire diameter is reduced to lower the stiffness thereof, it is more difficult to deliver sufficient energy for effective removal of the thrombosis. To overcome these disadvantages, miniature ultrasonic ablation tools have been developed, utilizing ultrasonic transducers sized for arterial insertion. While these devices overcome the

transmission wire difficulties, their small size severely limits the amount of ultrasonic energy available for direct mechanical action for fragmenting plaque and thrombosis and/or energy from improving diffusion and penetration of medicinal fluids as described in U.S. Patent No. 5,197,946.

Ultrasonic apparatus has also been utilized to assist in the delivery of medicaments in specific areas of a vein. For example, U.S. Patent No. 5,040,537 to Katakura teaches the use of injecting numerous fine capsules, with an agent being packed therein, into a patient's body and thereafter applying a shock wave to provide dominant positive pressure from outside the body to rupture the capsules dispersed in the body.

Thus, ultrasonic energy in the form of a pulsated shock wave is generated exterior to the body and imaged to selectively burst agent-containing capsules in order to selectively release the agent into the blood.

The present invention is directed to the discovery that ultrasound diagnostic media, particularly echo contrast agents containing microbubbles, utilized in conjunction with ultrasound, provides a safe and effective method for dissolving arterial thrombi without the use of thrombolytic drugs. Notably the ultrasound may be transcutaneously applied, applied by means of a transmission wire or generated intravascularly by means of a miniature ultrasonic tool. Moreover, a method in accordance with the present invention has been found to substantially enhance prior art treatments of thrombosis.

SUMMARY OF THE INVENTION

A method in accordance with the present invention utilizes the discovery of the effectiveness of applying a combination of ultrasonic energy and ultrasound imaging agents to dissolve arterial thrombi. Particularly, the present invention includes a method for substantially reducing and removing a thrombosis disposed within a body vessel by radiating an ultrasound imaging agent, particularly a microbubble containing echo contrast agent, proximate the thrombosis vessel with ultrasound. The ultrasound may be applied intravascularly, by means of a miniature ultrasonic transducer, or by a guide wire for transmitting ultrasound directly into the vessel, or transcutaneously by means of an external generator and transducer. Importantly, the introduction of a thrombolytic agent proximate the thrombosis further enhances the clot dissolution capability of a method in accordance with the present invention. This step is carried out during thrombolytic action by the thrombolytic agent on the thrombosis disposed within the body vessel.

This method is clearly distinguished from prior art techniques such as taught by Katakura in U.S. Patent No. 5,040,537, in which ultrasound generated exterior to the body vessel is used only to rupture capsules containing an active agent. Clearly, the prior art is specifically directed to the release of an active agent within a vessel, whereas the present invention is directed to introduction of a microbubble media that does not contain an active agent in order to enhance the effect of the ultrasound in removal of thrombosis and increase the effect of a thrombolytic agent during its activity in dissolving, or splitting up, a thrombus. The present invention involves a phenomena of long and short range ultrasound enhancement of inherent drug activity.

In accordance with one embodiment of the present invention, a selected dose of an echo contrast agent is injected into an occluded vessel, and ultrasonic energy is radiated from an external source into the echo contrast agent transcutaneously. It has been found that at certain frequencies of ultrasonic radiation, the thrombosis is substantially dissolved using this combination of steps. This embodiment of the present invention is based on the discovery that particular echo contrast agents, particularly those containing microbubbles, substantially increases the effectiveness of ultrasonic therapy in removing cardiovascular blockages, for example, fluorocarbon dispersions.

By way of specific example only, the echo contrast agent may be dodecafluropentane colloid dispersion, sold under the name of Echogen® by Sonus Pharmaceuticals, Inc. of Bothell, Washington, and the ultrasound may be introduced at a frequency of between about 24 kHz and about 53 kHz.

Thus, one embodiment of the present invention includes the step of introducing an echo contrast agent alone, proximate the thrombosis, and subsequently directing ultrasound into the thrombosis and proximate the echo contrast agent, in order to substantially dissolve the thrombosis without the use of thrombolytic agents.

The present invention also encompasses the enhancement, or acceleration, of the activity of a thrombolytic agent, and in that regard includes the steps of introducing a selected dose of an echo contrast agent and thrombolytic agent, proximate to a thrombosis disposed in the vessel of a body and radiating the thrombosis with ultrasound in order to effect removal of the thrombosis in less time than required by activity of the selected dose of thrombolytic agent alone. Thus, the introduction of an

echo contrast agent proximate the thrombosis has been found to enhance the effectiveness of both the ultrasound and the thrombolytic agent in removing the thrombosis.

More particularly, in accordance with the present invention, the thrombolytic agent introduced may be a any agent having suitable activity, such as, for example, streptokinase, staphlokinase, urokinase or a tissue plasminogen activator (TPA). These agents are set forth herein only by way of example and it should be appreciated that, as hereinabove recited, any thrombolytic agent may be utilized in accordance with the present invention. Notably, the amount of streptokinase introduced may be in low concentrations, for example, of less than 2000 μ/ml.

The present invention therefore also encompasses a method for removing a cardiovascular obstruction and in that regard includes the steps of delivering an echo contrast agent, alone or in combination with a thrombolytic agent, proximate a cardiovascular obstruction disposed in a vessel within a body, and directing ultrasound at the cardiovascular obstruction with proximate agents, of sufficient energy to remove the cardiovascular obstruction form the vessel.

Another embodiment of the present invention comprises the use of a miniature ultrasound transducer inserted intravascularly proximate the thrombosis, such as the transducer disclosed in U.S. Patent No. 5,269,291, said being incorporated herein *in toto* by this specific reference thereto. Thus, a method in accordance with this particular embodiment comprises introducing an echo contrast agent proximate the thrombosis and subsequently radiating the thrombosis and surrounding vessel with ultrasound transmitted internally from a miniature ultrasound transducer tip.

It should be appreciated that another workable method of transmitting ultrasound is by means of a catheter, or guide wire, inserted intravascularly to a position proximate the thrombosis, in order to transmit ultrasound along the wire from an external source.

Additionally, the radiation by ultrasound may include continuous or pulsed radiation.

In conjunction with the hereinabove enumerated method defining the present invention, also encompassed is an apparatus for the removal of a cardiovascular blockage which, in combination, includes ultrasonic means for radiating a cardiovascular blockage disposed within a body vessel and means for introducing a selected dose of an echo contrast agent proximate the cardiovascular blockage, in order to enhance the effect of the ultrasound in removing the cardiovascular blockage.

Clearly, the prior art teaches away from this discovery since prior art workers only were able to obtain enhancement for release of thrombolytic drugs within a vessel by introduction of ultrasound alone, which was thought to be due to mechanical agitation of surrounding vessel walls, as pointed out by Tachibana in U.S. Patent No. 5,197,946. It must be accepted that the mechanism taught by the Tachibana reference is not applicable to the present discovery in which it has been found that the introduction of an echo contrast agent proximate thrombosis, and subsequent ultrasonic radiation of the agent and thrombosis, substantially dissolve the thrombi, with or without the use of thrombolytic agents.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantages and features of the present invention will be better understood by the following description

when considered in conjunction with the accompanying drawings in which:

Figure 1 is a diagram of ultrasonic surgical apparatus in accordance with the present invention suitable for removing a thrombosis;

Figure 2 is a representation of an aorta having bilateral thrombosis induced in iliofemoral arteries;

Figure 3 is a representation similar to that shown in Figure 2 after one of the thrombi in the iliofemoral arteries has been removed in accordance with the apparatus and method of the present invention;

Figure 4 illustrates the effect of ultrasound frequency and addition of Albunex® or Echogen® on clot weigh reduction showing the lower frequency (24.8 kHz) of ultrasound was more effective than the higher frequency (53.3 kHz) in clot disruption. Echogen® significantly increased ultrasound clot disruption rate at 53.3 kHz and at 24.8 Khz, but Albunex® did not. USD indicates ultrasound, *; $p<0.01$ vs. no ultrasound with corresponding solution; **; $p<0.01$ vs. 53.3 kHz with corresponding solution #; $p<0.01$ vs. saline and Albunex® at the same frequency;

Figure 5 is a photograph of saline, Albunex® and Echogen® solution in transparent beaker before, 10 seconds and 3 minutes after ultrasound exposure at 24.8 kHz of frequency; Albunex® and Echogen® solutions were both whitish before ultrasound exposure; after 10 seconds of ultrasound exposure, Albunex® solution turned transparent; however, the appearance of the Echogen® solution did not change even after 3 minutes of ultrasound exposure; and

Figure 6 contains photomicrographs of Albunex® and Echogen® solution before, 10 seconds and 3 minutes after ultrasound exposure at 24.8 kHz of frequency (400x); after 10 seconds of ultrasound exposure, most of the Albunex® microbubbles disappeared; in contrast, no change was observed in Echogen® microbubbles even after 3 minutes of ultrasound exposure.

DETAILED DESCRIPTION

Turning now to Figure 1, there is shown apparatus 10 in accordance with the present invention for both enhancing the effectiveness of ultrasound in removing a thrombosis and for enhancing thrombolytic action of a thrombolytic agent which may include a vial 12 of a selected dose of an echo contrast agent alone or in combination with a thrombolytic agent, which, by way of a valve 14 and a catheter 16, provides a means for injecting, introducing and delivering the agents to a vessel 18 within a body 20 proximate a thrombosis 22 illustrated by the dashed lines in Figure 1.

The thrombolytic agent may comprise any suitable thrombolytic agent, for example, streptokinase. The type of echo contrast agent that is preferably used will be discussed in greater detail hereinafter.

Alternatively, the agents can be introduced or injected into the vessel 18 proximate the thrombosis 22 in any conventional manner, including, for example, hypodermic needle or the like (not shown).

Also shown in Figure 1 is a transducer 28 having a tip positioned exterior to the body 12 and interconnected to an oscillator/driver 34 which provides means for radiating the cardiovascular blockage 22 with ultrasound in order to effect removal thereof. In this embodiment of the present

invention, the ultrasound is transmitted transcutaneously, and thus the step of radiating ultrasound is a "noninvasive procedure".

The ultrasonic transducer 28 may be of any conventional design with a frequency range of about 1 kHz to about 1 mHz, the frequency being manually adjustable for transmitting ultrasonic frequency through the tip 30. As hereinafter set forth the frequency range is preferably limited to less than about 100 kHz, and more preferably between about 60 kHz and about 20 kHz.

The tip 30 provides means for coupling the ultrasound through a body surface 36, thus enabling transcutaneous, or transdermal, application of the ultrasound. It should be appreciated that the tip 30 can include means for focusing the ultrasound as may be required in order to concentrate or specifically direct the ultrasound to a desired area or volume.

The driver 34 is powered through conventional 110 volt line 38 and may have a power output of up to, for example, about 50 watts through a tip active area of about 0.75 inches by 0.75 inches. The power levels obtainable from the ultrasonic transducer 28 are capable of producing violent cavitation in tap water at atmospheric pressure and the limiting factor of introducing ultrasonic power into the body 20 would be possible skin irritation despite the output capability of the device. The driver 34 and transducer may be operated at a duty cycle of 100%, i.e., continuous output, or pulse-operated at, for example, a 50% duty cycle.

Alternatively, ultrasound may be transmitted intravascularly, rather than transcutaneously, as hereinabove described. For example, a miniature ultrasonic transducer 40, such as the device described in

U.S. Patent No. 5,269,291, incorporated herein by reference, may be utilized as a means for transmitting ultrasonic energy directly into and proximate the thrombosis 22 and surrounding vascular fluid. The miniature ultrasonic transducer 40 may be inserted into the vessel 18 by means of catheter 16.

It should be appreciated that ultrasound may be generated from driver 34 and transmitted therefrom via guide wire (not shown) directly into the vessel 18, as is well known in the art.

The present invention includes the introduction of an echo contrast agent, by means of the vial 12 or in other conventional manner, into the vessel 18 at a position proximate the thrombosis 22 and radiating ultrasound into the thrombosis 22. It has been found that the introduction of an echo contrast agent, in combination with the ultrasonic energy radiated into the site of the thrombosis, will substantially increase the effectiveness of ultrasound in removing the thrombosis. This embodiment provides for substantial dissolution of the thrombosis without the need for the introduction of thrombolytic agents.

Importantly, it has been found that when ultrasound is applied at a lower, rather than a higher, frequency, the effectiveness of the method is markedly enhanced. More particularly, when ultrasound is applied at about 100 kHz, or less, and even more particularly between approximately 25 kHz and approximately 53 kHz, the dissolution of thrombi is most significant. For example, at the frequency of about 53 kHz, the synergistic effect of a combination of ultrasound and echo contrast agent was most evident when compared to utilizing ultrasound alone.

Preferably, the echo contrast agent is comprised of a microbubble medium. More particularly, the microbubble medium may comprise a fluorocarbon colloidal suspension, and more preferably, a colloidal suspension of dodecafluropentane, for example, an agent presently marketed under the trademark Echogen® (Sonus Pharmaceuticals, Inc., Bothell, Washington) in which the microbubbles are stable to radiation by ultrasound. This is to be contrasted with microbubbles such as sonicated human serum albumin, an aqueous solution, Albunex® (Mallinckrodt, Medical, Inc., St. Louis, Missouri), which is unstable at low frequency ultrasound radiation.

It is important to recognize that control experiments, which tested the effect on blood clots of an echo contrast agent, without ultrasound, have shown in absence of significant clot dissolution. It has also been found that high intensity, low frequency ultrasound does have an effect on clot dissolution. Importantly, a method in accordance with the present invention utilizes the surprising discovery that a combination of echo contrast agent and ultrasound provides for effectively reducing or removing a thrombosis in less time than required by ultrasound radiation of the thrombosis without the use of said echo contrast agent. This may be due to the effect of microbubbles within the echo contrast agent that, when combined with ultrasonic energy, leads to increased cavitation of vascular fluid surrounding the thrombosis.

The following example demonstrates the effectiveness of the present invention and suitable/unsuitable microbubble media.

Example

Clot Preparation

Whole blood was obtained by antecubital venipuncture from three health volunteers in the morning after fasting 12 hours. The blood was allowed to coagulate in a glass test tube at room temperature for 2 to 3 hours. After aspirating serum, the clots were cut into pieces with weights ranging from 417 to 543 mg. Each clot was weighed on a precision scale (Menier PN 323, Princeton, New Jersey) and put in a plastic cassette with multiple holes, which are usually used for fixation of the pathologic specimen, to prevent each clot from the potential thrombus disrupting effects of floating under ultrasound exposure and also to keep the clot in the focus field of ultrasound.

External Ultrasound System

The external ultrasound system consists of an ultrasound generator (ENI generator EGR-700, Blackstone Ultrasonics, Jamestown, New York) and a transducer beaker. This system operates in continuous mode at a power output of 2.9 w/cm^2 with 2 different frequencies of 24.8 kHz and 53.3 kHz when the beaker is filled with the proper amount of liquid appropriate for each frequency.

Microbubbles (Echo Contrast Agents)

Two kinds of echo contrast agents, Albunex®, which is commercially available, and Echogen®, which is under clinical investigation, were used to examine their enhancing effect of ultrasound clot disruption. Albunex® is commercially manufactured sonicated human serum albumin microbubbles containing air within the albumin shell and Echogen® is a dodecafluropentane (DFP) solution which makes phase shift at body temperature from liquid to microbubbles. Other fluorocarbons with similar properties may also be used. 10 ml of Albunex® and 3 ml of Echogen®

were diluted with 1000 ml of warmed saline (1 and 0.3 volume percent respectively) and an appropriate amount of these solutions was used to fill the transducer beaker. The concentration of Albunex® and Echogen® solutions in this *in vitro* study were approximately 2 to 3 time higher than those for clinical use, assuming the blood pool of an average human is about 8% of the body weight.

Clot Disruption Protocol

Nine groups of clots, each of which consists of 8 clots, were examined. The first three groups of clots were placed in saline, Albunex®or Echogen® solution, respectively, but not exposed to ultrasound and just incubated for three minutes. The second three groups of clots were also placed in each solution respectively and exposed to 53.3 kHz of ultrasound for three minutes. The last three groups of clots were also placed in each solution respectively and exposed to 24.8 kHz of ultrasound for three minutes. Each plastic cassette containing a clot was placed in a transducer beaker filled with each solution, and exposed to ultrasound or not. The temperature of the solutions in the transducer beaker was maintained between 35° to 38°C. After external ultrasound exposure, each clot was weighted on the specimen scale again. Absolute reduction and per cent reduction in clot weight after each procedure were calculated as the extent of clot disruption.

Measurement of Particulate Size and Number

For measurement of number and size of particulate debris after ultrasound clot disruption, the solution containing the dissolved clots was collected after each procedure and stored at 4°C for 24 hours. A flow cytometric analyzer (Technicon H-I, Instruments Corp.) was used for the measurements. In brief,, fluid containing the particulates was aspirated directly and calibrated according to the laboratory protocol, which included

commercial control material. When the particulates passed through a laminar stream flow, the number and volume (V) of the particulates were determined by the measurement of laser light (R & D System Corp.). Particle size (diameter, d) was calculated using the formula $V = \pi d^3/6$.

Effect of Low Frequency Ultrasound on Microbubbles

Microbubbles of Albunex® and Echogen® solution without clot were observed macroscopically in a transparent beaker and under microscopy before, 10 seconds and 3 minutes after ultrasound exposure. Gross photographs and photomicrographs of each solution were taken for each specimen.

Statistical Analysis

All data were expressed as mean ± standard deviation. Percent reductions of clot weight after each procedure were compared using one-way ANOVA with Student-Newmann Keuls test as the post-hoc test. A p value of less than 0.05 was considered statistically significant.

Results

Effect of Low Frequency Ultrasound Exposure on Clot Disruption

The percent clot weight reductions in saline were 10 ± 6% after 3 minute of incubation without ultrasound, 22 ± 6% after 3 minutes of ultrasound exposure at 53.3 kHz and 72 ± 18% after 3 minutes of ultrasound exposure at 24.8 kHz as shown in Table 1 and Figure 4. Clot weight reductions were significantly greater after ultrasound exposures (no ultrasound vs. 53.3 kHz of ultrasound, p0.01, no ultrasound vs. 24.8 kHz of ultrasound, p0.01) and ultrasound exposure at 24.8 kHz was more effective compared to higher frequency of ultrasound at 53.3 kHz ($p<0.01$).

Effect of Microbubbles (Echo Contrast Agents) on Ultrasound Clot Disruption

Additional effects of echo contrast agents, Albunex® and Echogen®, on ultrasound clot disruption are summarized in Table 1 and Figure 1.

Without ultrasound exposure, the clots lost 10 ± 6% of their weight during 3 minutes of incubation in saline. However, Albunex®and Echogen®did not enhance clot weight reduction (6 ± 5% in Albunex® solution and 7 ± 5% in Echogen® solution after 3 minutes of incubation without ultrasound.

After 3 minutes of ultrasound exposure at 53.3 and 24.8 kHz, clot disruption was not accelerated in Albunex® solution compared to saline (26 ± 8% weight reduction in Albunex® solution vs. 22 ± 6% in sale at 53.3 kHz, p=ns, and 69 ± 25% weight reduction in Albunex® solution vs. 72 ± 18% in sale at 24.8 kHz; p=ns). However, Echogen® markedly enhanced ultrasound clot disruption at 53.3 and 24.8 kHz. After 3 minutes of ultrasound exposure at 53.3 kHz, the clot disruption rate was significantly $p<0.01$) augmented from 22 ± 6% in saline to 79 ± 15% in Echogen® solution. After 3 minutes of ultrasound exposure at 24.8 kHz, Echogen® significantly ($p<0.01$) accelerated clot disruption rate from 72 ± 18% in saline to 98 ± 4%, which is a nearly complete disruption of whole clots with a mean weight of 490 mg.

Particle Size After Ultrasound Clot Disruption

The average diameter of clot debris after 3 minutes of ultrasound exposure at 24.8 kHz of frequency was 3.3 μ (range 2.8 - 3.8) in saline, 4.3 μ (range 4.0 - 4.5) in Albunex® solution and 3.6 μ (range 3.0 - 4.2) in Echogen® solution. These particle sizes are smaller than those of red blood cells.

Effect of Ultrasound on Echo Contrast Agent Microbubbles

Figure 5 shows the photographs of saline, Albunex® and Echogen® solution before, 10 seconds and 3 minutes after ultrasound exposure at 24.8 kHz of frequency. Albunex® and Echogen® solutions were both whitish before ultrasound exposure, suggesting a large number of microbubbles present in each solution. Even after 3 minutes of ultrasound exposure, the appearance of the Echogen® solution did not change. However, the Albunex® solution turned from whitish to transparent after 10 seconds of ultrasound exposure, suggesting that most of Albunex® disappeared within 10 seconds of ultrasound exposure.

To confirm these observations, Albunex® and Echogen® solutions were examined under light microscopy. Figure 6 shows the photomicrographs of Albunex® and Echogen® microbubbles before, 10 seconds and 3 minutes after ultrasound exposure at 24.8 kHz of frequency corresponding to the photographs in Figure 5. After 10 seconds of ultrasound exposure, most of the Albunex® disappeared, and only a few normal bubbles could be identified. In contrast, no change was observed in Echogen® microbubbles even after 3 minutes of ultrasound exposure.

This study demonstrates that low frequency (24.8 and 53.3 kHz) ultrasound dissolves a substantial amount of clot without a thrombolytic agent. Furthermore, Echogen® microbubbles markedly enhance ultrasonic clot disruption, whereas Albunex® microbubbles under these frequencies *in vitro* do not. The majority of Albunex® microbubbles disappeared or were destroyed after 10 seconds of low frequency ultrasound exposure. However, there was no apparent reduction in Echogen® microbubbles even after 3 minutes of ultrasound exposure. This difference in microbubble characteristics in the acoustic field might be responsible for the different efficacy in enhancement of ultrasound clot disruption.

The predominant mechanism of external (remote) low frequency ultrasound clot disruption is believed to be acoustic cavitation (unstable or transient cavitation) and precavitation (stable cavitation) as well as contribution of microstreaming and microcurrents generated under acoustic pressure field. Cavitation (unstable or transient cavitation) is the formation and collapse of the microscopic bubbles when ultrasound waves pass through a liquid with an alternating pressure. These bubbles or cavities take some cycles to grow, and then collapse violently at the point called resonant size, which is larger at lower frequency, and collapse of larger bubbles generate more energy compared to smaller bubbles. Precavitation (stable cavitation) is the oscillation or vibration of microbubbles present or formed in the acoustic field. it generates large shock waves and also causes microstreaming of the liquid.

In this study, Echogen® microbubbles, which were resistant to ultrasound irradiation, enhanced ultrasound clot disruption; whereas Albunex® microbubbles, which were fragile and vulnerable to low frequency ultrasound exposure, did not affect the rate of ultrasound clot disruption. These findings are different from previous findings which demonstrated enhancement of clot dissolution by addition of Albunex® microbubbles. This discrepancy might be due to the difference in the experimental settings. They studied the synergetic effect of Albunex® microbubbles with urokinase for 30 to 120 minutes using 170 kHz of ultrasound frequency. If Albunex® microbubbles do not disappear by ultrasound exposure at 170 kHz, it might enhance combined effect of ultrasound and urokinase in clot dissolution. The present discovery is that collapse of Albunex® microbubbles does not generate enough energy to enhance ultrasound clot disruption, and that Echogen® microbubbles enhanced ultrasound clot disruption by oscillation of Echogen®

microbubbles (precavitation) and/or formation of new Echogen® microbubbles and their collapse (cavitation).

Table 1.

Effect of Ultrasound Frequency and Echo Contrast Agents, Albunex® and Echogen®, on Clot Disruption

USD Frequency (kHz)	Solution	Pre USD Clot Weight (mg)	Post USD Clot Weight (mg)	Disrupted Clot Weight (mg)	% Reduction in Clot Weight (%)
No	Saline	472±26	427±44	45±25	10±6
No	Albunex®	490±31	461±34	29±26	6±5
No	Echogen®	488±17	456±23	33±26	7±5
53.3	Saline	470±44	364±39	106±32	22±6*
53.3	Albunex®	446±26	330±53	116±30	26±8*
53.3	Echogen®	464±36	97±72	367±72	79±15*#
24.8	Saline	484±43	143±97	340±61	72±18**
24.8	Albunex®	451±31	144±127	307±101	69±25**
24.8	Echogen®	490±30	9±19	481±31	98±4**#

* $p<0.01$ vs. no ultrasound with corresponding solution
** $p<0.01$ vs. 53.3 kHz with corresponding solution
\# $p<0.01$ vs. saline and Albunex® at the same frequency

Although there has been hereinabove described a specific arrangement of ultrasonic apparatus and a method for thrombi dissolution in accordance with the present invention, for the purpose of illustrating the manner in which the invention may be used to advantage, it should be appreciated that the invention is not limited thereto. Accordingly, any and all modifications, variations, or equivalent arrangements which may occur to those skilled in the art, should be considered to be within the scope of the present invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A method for removing a thrombosis, said method comprising the steps of:

(a) radiating a thrombosis disposed in a vessel within a body, with ultrasound in order to effect removal of the thrombosis; and

(b) during the radiation, introducing a selected dose of an echo contrast agent causing enhancement of the ultrasound in removing of the thrombosis, said echo contrast agent being stable to the radiation of ultrasound.

2. The method according to claim 1 wherein the ultrasound is applied transcutaneously.

3. The method according to claim 1 wherein the ultrasound is applied intravascularly.

4. The method according to claim 1 wherein the echo contrast agent is comprised of a microbubble medium.

5. The method according to claim 4 wherein the microbubble medium comprises a fluorocarbon colloidal suspension.

6. The method according to claim 4 wherein the microbubble medium is a colloidal suspension comprising dodecafluropentane.

7. The method according to claim 1 wherein the ultrasound is introduced at a frequency of below 100 kHz.

8. The method according to claim 7 wherein the ultrasound is introduced at a frequency of below about 60 kHz.

9. The method according to claim 8 wherein the ultrasound is introduced at a frequency of about 20 kHz.

10. A method for enhancing thrombolytic action of a thrombolytic agent, said method comprising the steps of:

(a) radiating a thrombosis, disposed within a vessel within a body, with ultrasound in order to effect removal of the thrombosis;

(b) introducing a selected dose of a thrombolytic agent proximate the thrombosis; and

(c) introducing a selected date of an echo contrast agent proximate the thrombosis in order to enhance the effectiveness of both the ultrasound and the thrombolytic agent in removing the thrombosis.

11. The method according to claim 10 wherein the echo contrast agent is comprised of a microbubble medium, said microbubble medium being stable to the radiation of ultrasound.

12. The method according to claim 11 wherein the microbubble medium is a colloidal suspension comprising dodecafluropentane.

13. The method according to claim 11 wherein the thrombolytic agent is comprised of streptokinase.

14. Apparatus for removal of a cardiovascular blockage, said apparatus comprising, in combination:

ultrasonic means for radiating a cardiovascular blockage with ultrasound;

an echo contrast agent selected to cause enhancement of the ultrasound in removing the cardiovascular blockage; and

means for introducing a selected dose of the echo contrast agent proximate the cardiovascular blockage.

15. The apparatus according to claim 14 wherein the ultrasonic means includes means for applying ultrasound transcutaneously.

16. The apparatus according to claim 14 wherein the ultrasonic means includes means for applying ultrasound intravascularly.

17. The apparatus according to claim 14 wherein the echo contrast agent comprises a microbubble medium, said microbubble medium being stable to the radiation of ultrasound.

18. The apparatus according to claim 17 wherein the microbubble medium comprises a fluorocarbon colloidal suspension.

19. The apparatus according to claim 17 wherein the microbubble medium is a colloidal suspension comprising dodecafluropentane.

20. The apparatus according to claim 14 wherein the ultrasonic means is configured for radiating ultrasound at a frequency below about 100 kHz.

21. The apparatus according to claim 20 wherein the ultrasonic means is configured for radiating ultrasound at a frequency below about 60 kHz.

22. The apparatus according to claim 20 wherein the ultrasonic means is configured for radiating ultrasound at a frequency of about 20 kHz.

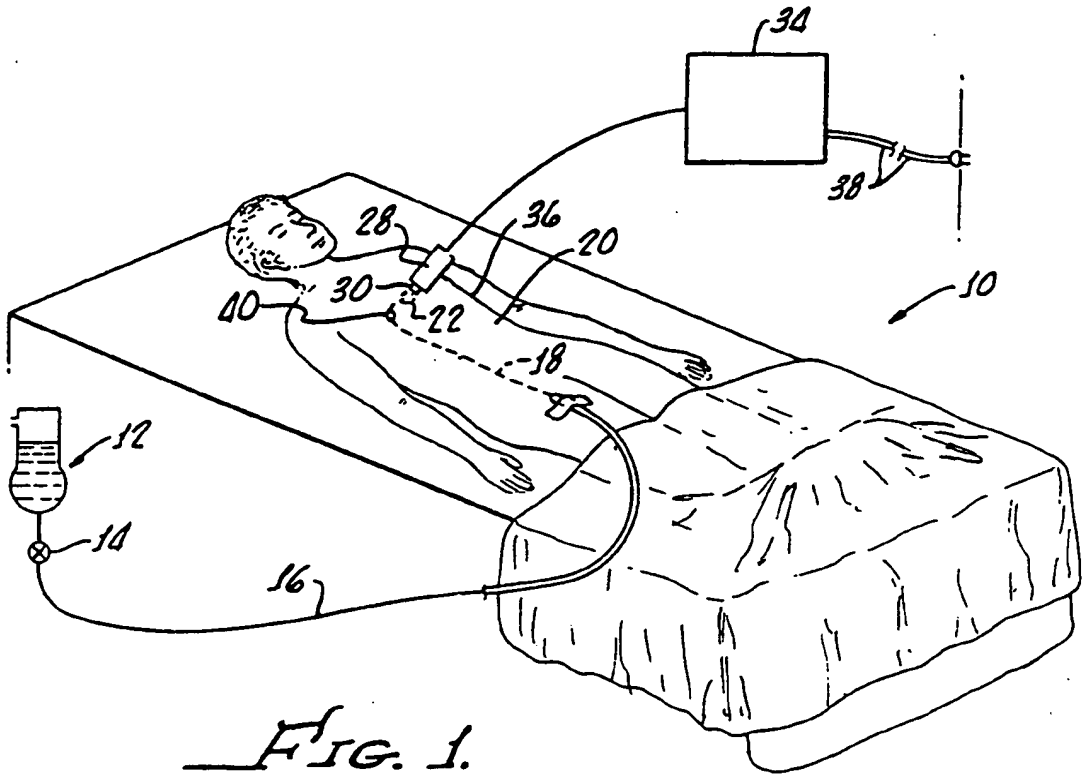

FIG. 1.

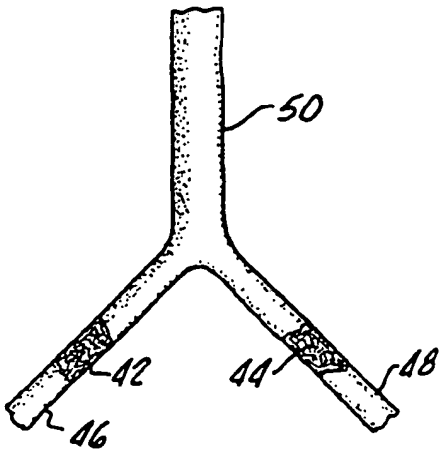

FIG. 2.

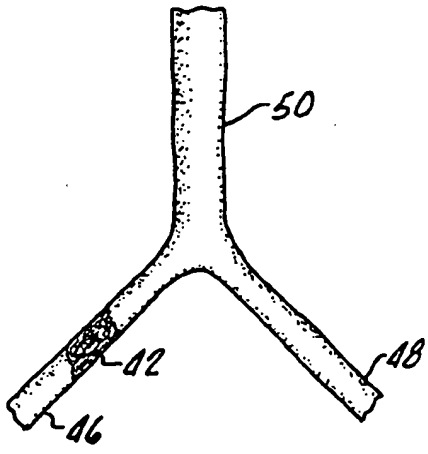

FIG. 3.

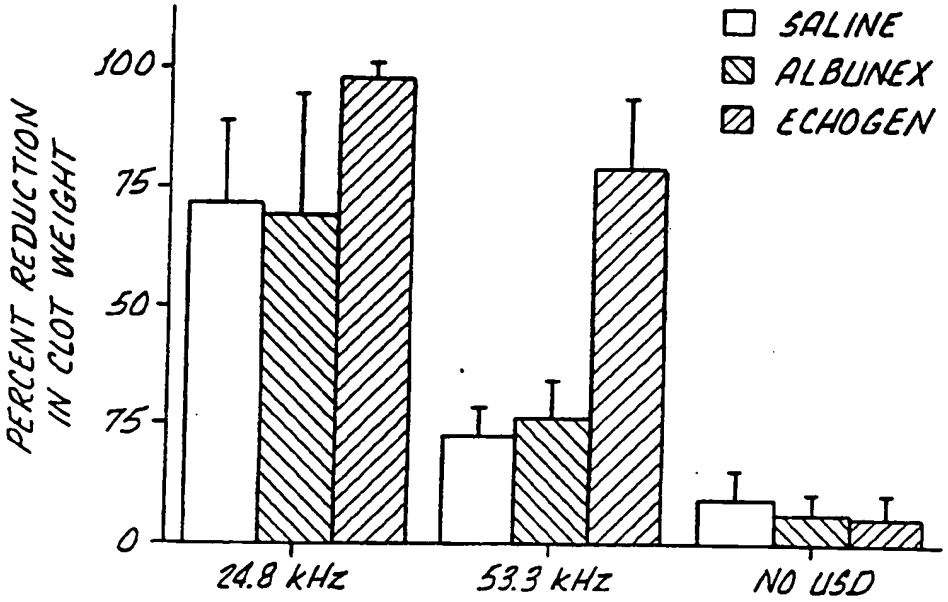
SALINE
ALBUNEX
ECHOGEN
PERCENT REDUCTION IN CLOT WEIGHT
100
75
50
75
0
24.8 kHz
53.3 kHz
NO USD

FIG. 4.

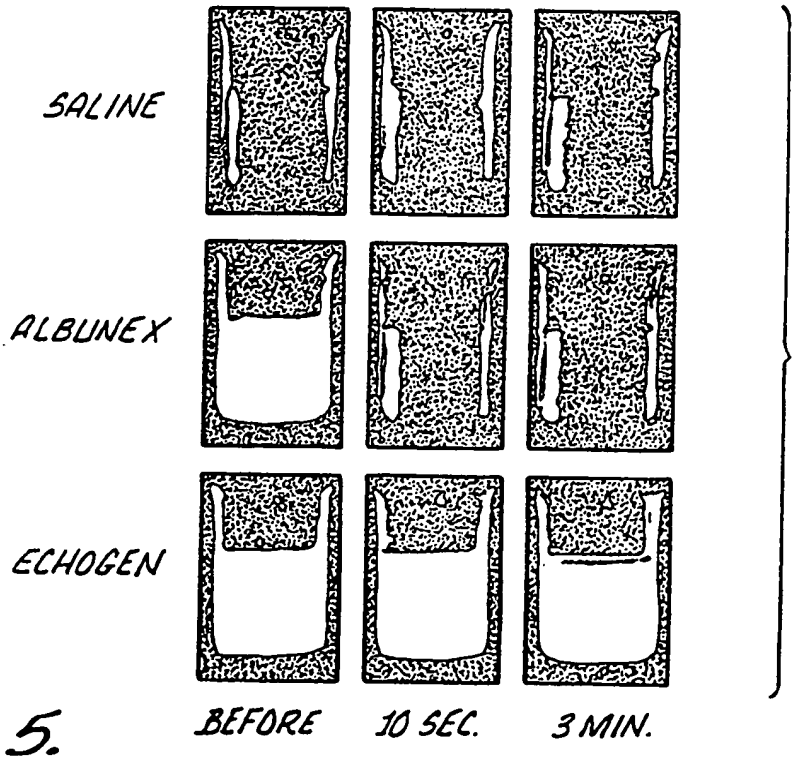
SALINE
ALBUNEX
ECHOGEN
BEFORE
10 SEC.
3 MIN.

FIG. 5.

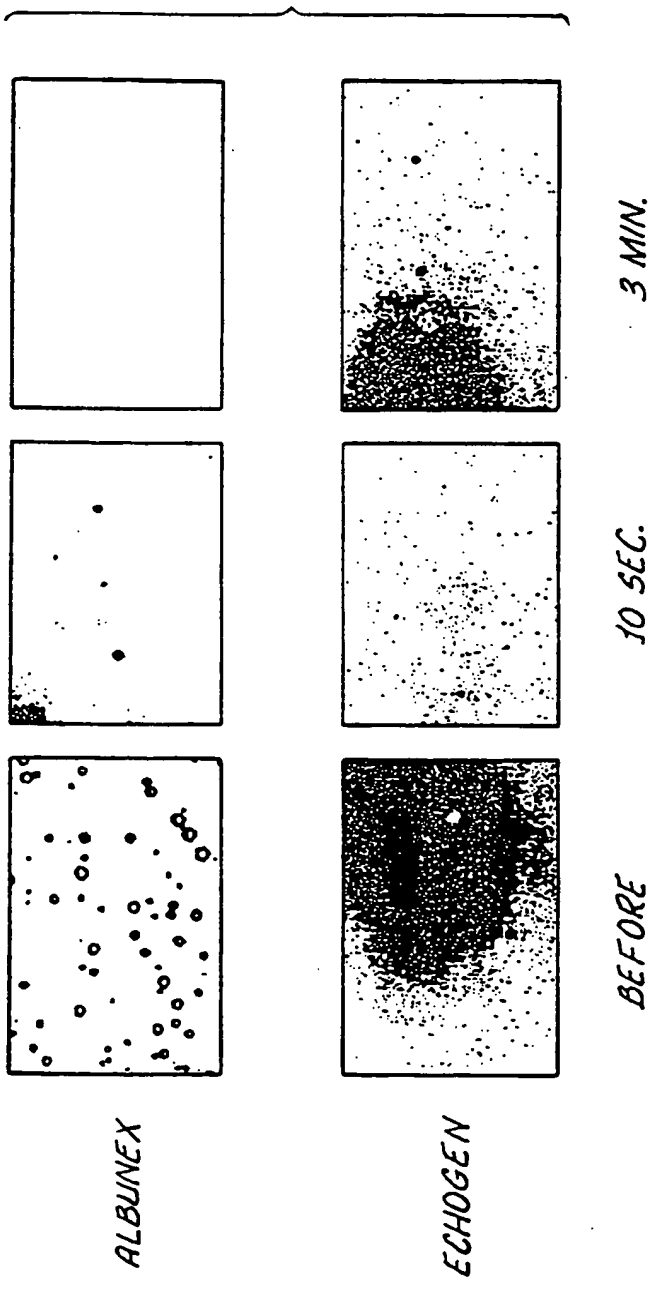

FIG. 6.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06652

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61B 17/20
US CL :604/22
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/653.4, 660.01, 660.03; 604/19-22, 49, 52, 53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

MERCK CHEMICAL INDEX

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 4,622,952 (GORDON) 18 November 1986, note column 5 lines 37-44.	1-4, 7-9 --- 4, 6, 14-22
X --- Y	US, A, 5,149,319 (UNGER) 22 September 1992, note column 6 line 56, column 7 lines 24-35, and column 7 line 65 to colum 8 line 2.	1-6 --- 7-22
Y	US, A, 5,405,318 (NITA) 11 April 1995, see entire reference.	14-22
Y	US, A, 5,399,158 (LAUER ET AL.) 21 March 1995, see entire reference.	10-13
A, P	US, A, 5,509,896 (CARTER) 23 April 1996	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 JUNE 1996	Date of mailing of the international search report 09 JUL 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHALIN SMITH Telephone No. (703) 308-2988

Form PCT/ISA/210 (second sheet)(July 1992)*

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG

Search Terms: utlrasound, thrombolysis, clot, radiation, contrast agent, stenosis